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Research Article

HEPATOPROTECTIVE ACTIVITY OF EXTRACTS FROM STEM OF *MUSSAENDA ERYTHROPHYLLA* LAM. AGAINST CARBON TETRACHLORIDE – INDUECD TOXICITY IN RATS

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ABSTRACT

*Mussaenda erythrophylla (*Rubiaceae) is native to western tropical Africa, occasionally seen in gardens and parks as ornamental plant in India. The Hepatoprotective activity of ethyl acetate and methanol extracts of *M.erythrophylla (ME)* Stem against carbon tetrachloride (CCl_4) induced liver damage in Wistar albino rats. Ethyl acetate and methanolic extracts of *ME* (100,200mg/kg.p.o.), were administerd respectively, Silymarin (25 mg/kg.p.o.) was given as reference standard. The stem extracts were effective in protecting the liver against the injury induced by CCl_4 in animals. This was evident from significant reduction in serum enzyme, SGOT, SGPT, ALP and Total bilirubin (TB).Various pathological changes like centribular necrosis and vacuolization were observed in CCL_4 treated rats, which were significant protective activity in groups treated with ME and silymarin. It was concluded from the study that ethyl acetate and methanolic extracts of ME possess hepatoprotective activity against CCl_4 induced hepatotoxicity in rats**.**

Keywords: Hepatoprotective,Carbon tetrachloride, *Mussaenda erythrophylla*, Silymarin.

INTRODUCTION

Mussaenda erythrophylla (Rubiaceae) is native to western tropical Africa, occasionally seen in gardens and parks as ornamental plant in India and is commonly known as mussenda (telugu), nagavalli (Sanskrit) and red flag bush (English)[1.] It is a perennial, evergreen shrub with branched tap root system. The roots are useful for cough, jaundice and when chewed acts as an appetizer. A number of triterpenoids and glycosides were reported. **mussaenda genus viz.,** contains mussaendosides U(1) and V(2)[2], mussaendosides G(1) and K(2) are two new triterpenoid saponins[3], mussaendosides A-C, M and N with cyclolanostene type aglycone [4-5] and aureusidin[6],iridoid glycosides[7]. The pharmacological activities reported from Mussaenda species were diuretic, antiphlogistic, antipyretic and effective in laryngopharyngitis, acute gastroenteritis and dysentery and also anti-fertility activity[8]. The evaluation of the stem of *M.erythrophylla* in the treatment of liver disease has not been reported in the laboratory animals. The present studies were performed to assess the hepatoprotective activity in rats against carbon tetrachloride as hepatotoxin to prove its claim in the folklore practices against liver disorders.

MATERIALS AND METHODS

PLANT MATERIAL

The stem of ***Mussaenda erythrophylla*** was procured from M.V.P colony, Visakhapatnam, in the month of April 2006, (Voucher number TSN/DOP/ME 0406). The authentification of the plant was done by prof. M. Venkaiah, Dept. of Botany, Andhra University, Visakhapatnam.

PREPARATION OF EXTRACT

Freshly collected plant material was shade dried at room temperature and coarsely powdered in Wiely mill. The powdered stem (1kg) was extracted successively with hexane, ethyl acetate and methanol using soxhlet apparatus. The crude extract was evaporated to dryness in a rotary film evaporator (Roteava,Equitron, Medica instrument, India) and found to be 2.5 gms, 30 gms and 25gms respectively. Preliminary phytochemical screening of ethyl acetate extract of *M. erythrophylla* stem revealed the presence of steroids, triterpenoids and flavonoids; methanol extract tested positive for glycosides, tannins and saponins.

The constituents present in the ethyl acetate and methanol extracts of *M.erythrophylla* stem initiated to carry out the hepatoprotective activity. The ethyl acetate and methanol extracts were subjected to hepatoprotective activity in rats. Silymarin was used as positive control at dose of 25mg/kg.p.o. All the test substances were suspended in vehicle i.e. 5% acacia mucilage. The extracts were tested for activity at doses of 100mg/kg and 200mg/kg.p.o.

DRUG AND CHEMICALS

CCl_4 was obtained from Poona Chemical Laboratory, Pune, India. Silymarin-Microlab, Bangalore, Karnataka, India. Estimation kits-Span Diagnostics, Surat, India. All other chemicals were obtained from local sources (Sai chemicals, Visakhapatnam) and were of analytical grade.

ANIMALS

`Wistar albino rats of either sex weighing between 200-250 gm were obtained from M/s. Mahavir Enterprises, Hyderabad. The animals were housed under standard environmental conditions (temperature of 22 ± 1°C with an alternating 12 h light – dark cycle and relative humidity of 60 ± 5%), one week before the start and also during the experiment as per the rules and regulations of the Institutional Ethics Committee and by animal regulatory body of the government (Regd: No: 516/01/A/CPCSEA). They were fed with standard laboratory diet supplied by M/s. Rayans biotechnologies Pvt. Ltd., Hyderabad, and water *ad libitum*

ACUTE TOXICITY STUDIES

Acute toxicity studies were performed for extracts according to the toxic classic method as per OECD guidelines[9]. Female albino rats were used for the acute toxicity study. The animals were kept fasting overnight providing only water, after which the extracts were administered orally at the dose of 300 mg/kg and observed for 14 days. If mortality was observed in 2 out of 3 animals, then the dose administered was assigned as toxic dose. If the mortality was observed in 1 animal, then the same dose was repeated again to confirm the toxic dose. If the mortality was not observed, the procedure was repeated for further higher dose i.e., 2000 mg/kg, 2500mg/kg, 3000mg/kg.

CARBONTETRACHLORIDE-INDUCED HEPATOTOXICITY

The animals were divided into seven groups of six animals each. Group-I served as normal control received 5% acacia mucilage (1 ml/kg.p.o) daily once for 7 days. Group-II served as toxic control and received CCl_4 (0.5 ml/kg i.p) daily once for 7 days[10]. Group-III was treated with the reference drug Silymarin (25 mg/kg .p.o) and followed by CCl_4 (0.5 ml/kg i.p) daily once for 7 days[11]. Groups IV-V were treated with methanol extract of *M.erythrophylla* stem at doses of 100 and 200 mg/kg p.o. in acacia mucilage respectively followed by CCl_4 (0.5 ml/kg i.p) daily once for 7 days. Groups VI-VII were treated with ethyl acetate extract of *M.erythrophylla* stem at doses of 100 and 200 mg/kg p.o, in acacia mucilage respectively followed by CCl_4 (0.5 ml/kg i.p) daily once for 7 days. After completion of treatment blood was collected, serum was separated and used for determination of biochemical parameters.

COLLECTION OF BLOOD SAMPLES

All the animals were sacrificed on 7th day under light ether anesthesia.The blood samples were collected separately in sterilized dry centrifuge tubes by puncture retro-orbital plexes and allowed to coagulate for 30 min at 37 °C **.**The clear serum was separated at 2500 rpm (Microcentrifuge) for 10min and subjected to biochemical investigation viz..,serum glutamic oxaloacetate transe aminase (SGOT), serum glutamic Pyruvate transe aminase (SGPT), Alkaline phosphatase (ALP) and Total Bilirubin (TB).

ASSESSMENT OF LIVER FUNCTION

The Serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) were estimated by UV kinetic method in which both SGOT and SGPT were assayed based on enzyme-coupled system; where keto acid formed by the aminotransaminase reacts in a system using NADH.The coenzyme is oxidized to NAD and the decrease in absorbance at 340 nm for SGOT malate dehydrogenase (MDH) reduces to malate with simultaneous oxidation of NADH to NAD.The rate of oxidation of NADH is measured, where as SGPT [12] the pyruvate formed in the reaction is converted to lactate by lactate dehydrogenase.Estimation of Alkaline phosphate (ALKP)[13] involves hydrolysis of P-nitrophenyl phosphate by alkaline phosphatase to give P-nitrophenol, which gives yellow color in alkaline solution. The increase in absorbance due to its formation is directly proportional to alkaline phosphate (ALKP) activity. Estimation of total bilirubin (TB) [14] involved the reaction of bilirubin with diazotized sulphanic acid to form an azocompound, the color of which is measured at 546 nm .All the estimations were carried out using standard kits in semi auto analyzer Screen Master 3000.

HISTOPATHOLOGICAL STUDIES

The isolated liver specimens were trimmed to small pieces and preserved in formalin (10%) solution for 24 hrs. The liver specimens were subjected to dehydration with acetone of strength 70, 80, 100 % respectively, each for one hour. The infiltration and impregnation was done by treatment with paraffin wax twice each time for one hour. Specimens were cut into sections of 4-6 µm thickness and were stained with haemotoxylin and eosin (H-E) and later the microscopic slides of the liver were photographed in light microscope (Axiostar plus).

STATISTICAL ANALYSIS

Results of biochemical estimation were reported as mean ±SEM for determination of significant inter group difference was analysed separately and one-way analysis of variance (ANOVA) was carried out[15].Dunnet's test was used for indidual comparisons[16].

RESULTS

The LD_{50} of ethyl acetate extract and methanol soluble extracts were found to be 2000 mg/kg .b.w. 1/10th and 1/20th of these doses (200 mg/kg. b.w, and 100mg/kg. b.w) were selected for the evaluation of hepatoprotective activity.

considering the enzyme level difference between hepatotoxin treated and control rats as 100% of level of reduction and recorded in (Table- 2).The comparative efficacy of the extracts and silymarin tested for their hepatoprotective activity were depicted in the form of a bar diagram fig(1).

The effect of ethyl acetate and methanol extracts of *M.erythrophylla* stem on CCl_4 induced liver damage in rats with reference to biochemical changes in serum is shown in table.(1). Percentage decrease or increase was calculated by

Histopathology of liver tissues (a) Group I — section shows central vein surrounded by hepatic cord of cells (normal architecture). (b) Group II—section shows patches of liver cell necrosis with inflammatory collections, around central vein. (c) Group III—almost near normal. (d) Group IV—inflammatory collections around central vein and focal necrosis with sinusoidal dilatation. (e) Group V—less inflammatory cells around

central vein, absence of necrosis. (f) Group VI—less inflammation around dilated central vein. (g) Group VII—minimal inflammatory cellular infiltration. Almost near normal liver architecture. Regeneration of hepatocytes around central vein.

Table 1

Effect of different Extracts of of *M.erythrophylla* stem on carbon tetrachloride -induced toxicity in rats.

Groups	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	TB (mg/dl)
Control	123.83±1.70	82.66±1.54	158.33±2.20	1.06±0.08
CCL_4 0.5 ml/kg.b.w	997.66±22.74	736.0±17.57	488.48±8.23	4.48±0.17
Sylimarin 100mg/kg	223.16±1.40	164.50±1.60	185.16±2.80	1.7±0.13
MEME 100mg/kg	485.16±1.88	395.16±2.57	373.83±1.53	3.25±0.07
MEME 200mg/kg	429.33±0.88	314.83±1.19	315.5±1.25	2.63±0.13
EEME 100mg/kg	419.16±1.07	341.16±1.19	287.5±1.72	2.78±0.04
EEME 200mg/kg	365.66±1.20	285.66±1.33	245.33±1.22	2.33±0.08

EEME –Ethyl acetate extract of *Mussaenda erythrophylla*, MEME-Methanol extract of *Mussaenda erythrophylla*, Values are expressed in mean ± SEM, n=6, in each group. **Significant increase compared to Control (P≤0.01), ***Significant reduction compared to Control. (P≤0.01),

Table-2

Percentage decrease in levels of biochemical parameters due to treatment with different Extracts of of *M.erythrophylla* stem.

Treated with	% Decrease Biochemical Levels			
	SGOT	SGPT	ALP	TB
Silymarin 25mg/kg	83.63	87.47	91.87	81.28
MEME 100mg/kg	58.64	52.16	34.72	35.96
MEME 200mg/kg	65.03	64.46	52.39	54.09
EEME100mg/kg	66.20	60.43	60.87	49.70
EEME 200mg/kg	72.32	68.92	61.53	62.86

EEME –Ethyl acetate extract of *Mussaenda erythrophylla*, MEME-Methanol extract of *Mussaenda erythrophylla*

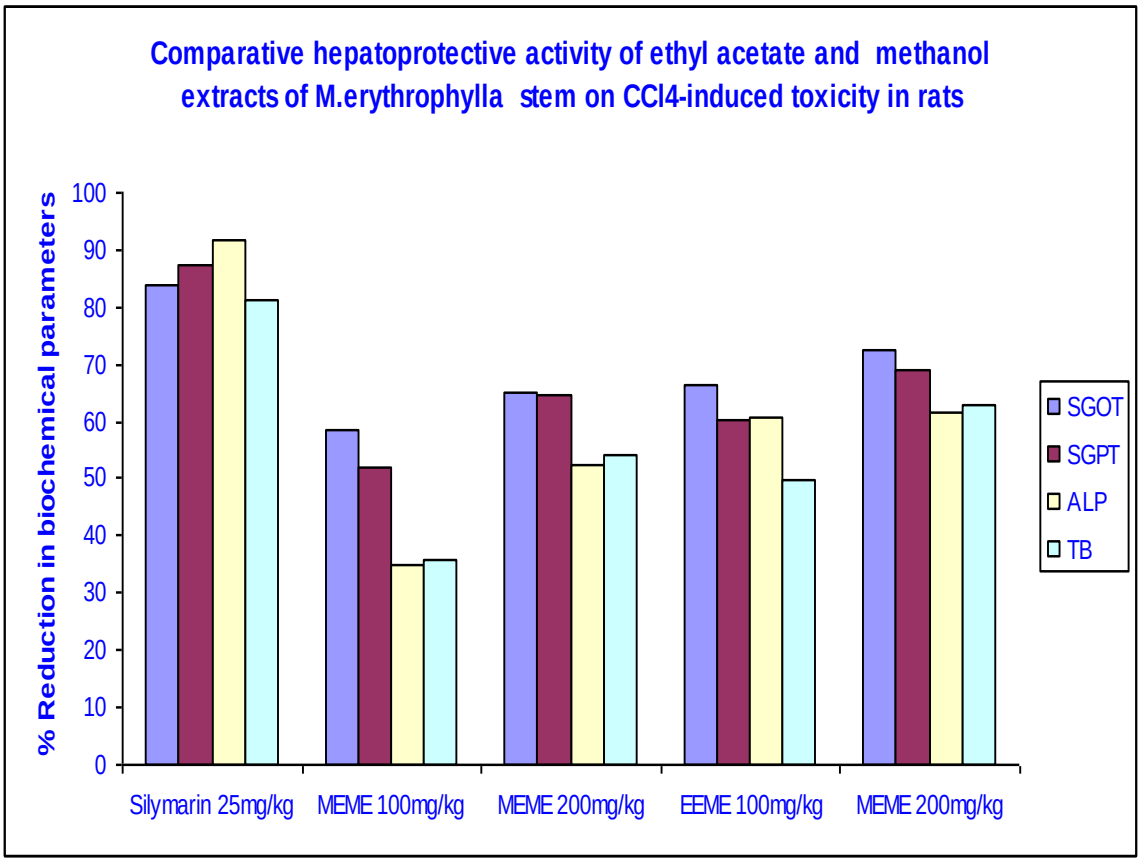
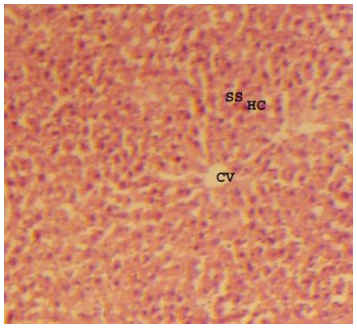
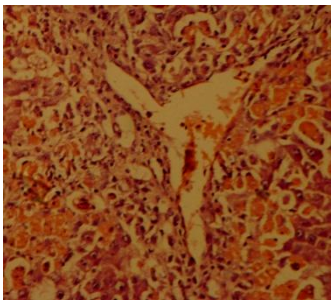

Comparative hepatoprotective activity of ethyl acetate and methanol extracts of M.erythrophylla stem on CCl4-induced toxicity in rats
% Reduction in biochemical parameters
100
90
80
70
60
50
40
30
20
10
0
Silymarin 25mg/kg
MEME 100mg/kg
MEME 200mg/kg
EEME 100mg/kg
MEME 200mg/kg
SGOT
SGPT
ALP
TB

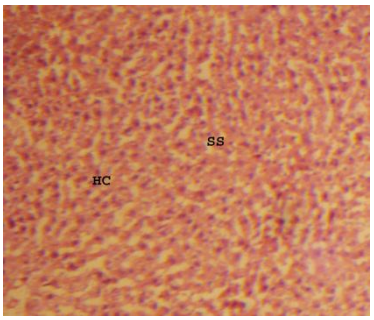
Figure (a) Representative photographs of histopathological changes showing effect of the test material on the rats intoxicated with carbon tetrachloride. a. normal control, b.Carbontetrachloride 0.5ml/kg.i.p., c. silymarin 25mg/kg.p.o., d,e. Methanolic extract (100mg/kg,200mg/kg.p.o.,), f,g. ethyl acetate extract (100mg/kg,200mg/kg..p.o.)



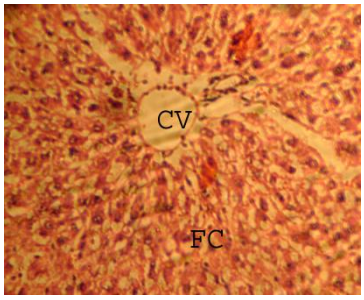
Fig(a)



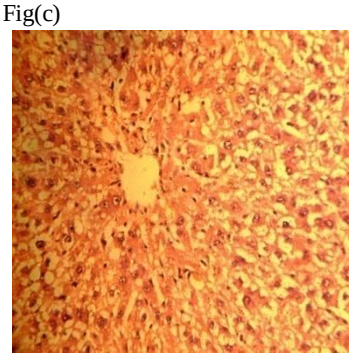
Fig(b)



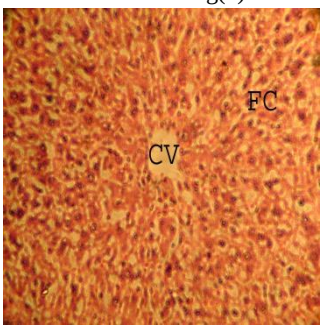
Fig(c)



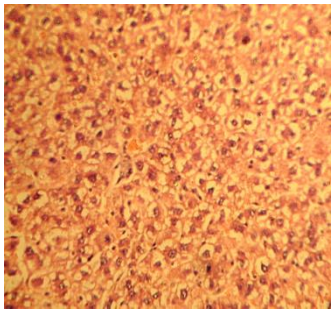
Fig(d)



Fig(e)



Fig(f)



Fig(g)

DISCUSSION AND CONCLUSION

The carbon tetrachloride mechanism begins with the trichloromethyl radical ($\cdot CCl_3$) by the action of the mixed function of cytochrome P-450 oxygenase system. This free radical, which is initially formed as unreactive, reacts very rapidly with oxygen to yield a highly reactive trichloromethyl peroxy radical ($\cdot OOCCl_3$). Both radicals are capable of binding to proteins, lipids or abstracting a hydrogen atom an unsaturated lipid, thus initiating lipid peroxydation. This proceses of lipid peroxidation can significantly damage hepatic plasma membranes[17].The increased levels of SGOT,SGPT,ALP and TB are conventional indicators of liver injury[18].The ability of hepatoprotective drug to reduce the injurious effect or to preserve the normal hepatic physiological mechanisms, that have been disturbed by a hepatotoxin, is the index of its protective effect[19]. Hepatocellular necrosis leads to evaluation of the serum marker enzymes, which are released from the liver in blood[20].The present study revealed a significant increase in the activities of SGOT,SGPT,ALP and TB levels on exposure to CCl_4 indicating considerable hepatocellular injury. Administration of both ethyl acetate extracand methanol extracts at two different dose levels attenuated the increased levels of the serum enzymes, produced by CCl_4 and caused a subsequent recovery towords normalization almost like that of silymarin treatment.

The hepatoprotective effect of the drugs was further concluded by the histopathological examinations of the liver sections which reveal that the normal liver architecture was distrurbed by hepatotoxin intoxication. In the liver sections of the rats treated with ethyl acetate extract and methnolic extract and intoxicated with CCl_4 the normal cellular architecture was retained as compared to silymarin, thereby confirming the protective effect of the extracts of *M. erythrophylla*.

Accordance with these results, ethyl acetate extract and methanolic extract at different dose levels offer hepatoprotection dose dependent activity. But Group VII (ethyal acetate extract 200mg/kg.b.w.p.o) is more effective than all other groups and it may be hypothesized that rich content of flvonoids may be responsible. The hepatoprotective activity of these drugs might be due to stabilization of the membrane inhibiting effect on lipid peroxidation or due to their stimulatory effects on hepatic regeneration. The protective action may be due to scavenging effect of free radicles. Hepatoprotective action of certain phytoconstituents like flavonoids[21-22], saponins[23,] triterpenoids[24], has been well documented in the literature.The author some phytoconstituents like 5- hydroxy-7, 4'-dimethoxy flavones, β-sitosterol,3- iso cumaryloxy – cyclopropane-1-oic acid,4 -hydroxy-3-methaxy cinnamic acid[25], was isolated from ethyl acetate extract of *M.erythrophylla* these phytoconstituents are alone or in combination responsible for hepatoprotective activity In conclusion this study confirms the therapeutic potential of stem of *M.erythrophylla.*

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